

## **IN THE SPECIFICATION**

Delete the third full paragraph on page 50 and replace it with:

Oil-emulsion compositions suitable for use as adjuvants in the invention include squalene-water emulsions, such as ~~MF59~~ MF59® (5% Squalene, 0.5% ~~Tween 80~~ TWEEN 80®, and 0.5% ~~Span 85~~ SPAN 85®, formulated into submicron particles using a microfluidizer). See WO90/14837. See also, Frey et al., "Comparison of the safety, tolerability, and immunogenicity of a ~~MF59-adjuvanted~~ MF59®-adjuvanted influenza vaccine and a non-adjuvanted influenza vaccine in non-elderly adults", Vaccine (2003) 21:4234-4237. ~~MF59~~ MF59® is used as the adjuvant in the FLUAD™ influenza virus trivalent subunit vaccine.

Delete the paragraph bridging pages 50 and 51 and replace it with:

Particularly preferred adjuvants for use in the compositions are submicron oil-in-water emulsions. Preferred submicron oil-in-water emulsions for use herein are squalene/water emulsions optionally containing varying amounts of MTP-PE, such as a submicron oil-in-water emulsion containing 4-5% w/v squalene, 0.25-1.0% w/v ~~Tween 80™~~ TWEEN 80® (polyoxyethylsorbite monooleate), and/or 0.25-1.0% Span 85.TM. (sorbitan trioleate), and, optionally, N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-s-n-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE), for example, the submicron oil-in-water emulsion known as "~~MF59~~ MF59®" (International Publication No. WO90/14837; U.S. Pat. Nos. 6,299,884 and 6,451,325, incorporated herein by reference in their entireties; and Ott et al., "MF59--Design and Evaluation of a Safe and Potent Adjuvant for Human Vaccines" in Vaccine Design: The Subunit and Adjuvant Approach (Powell, M. F. and Newman, M. J. eds.) Plenum Press, New York, 1995, pp. 277-296). ~~MF59~~ MF59® contains 4-5% w/v Squalene (e.g. 4.3%), 0.25-0.5% w/v ~~Tween 80™~~ TWEEN 80®, and 0.5% w/v Span 85.TM. and optionally contains various amounts of MTP-PE, formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, Mass.). For example, MTP-PE may be present in an amount of about 0-500 µg/dose, more preferably 0-250 µg/dose and most preferably, 0-100 µg/dose. As used herein, the term "MF59-0" refers to the above submicron oil-in-water emulsion lacking MTP-PE, while the term MF59-MTP denotes a formulation that

contains MTP-PE. For instance, "MF59-100" contains 100 µg MTP-PE per dose, and so on. MF69, another submicron oil-in-water emulsion for use herein, contains 4.3% w/v squalene, 0.25% w/v ~~Tween 80™~~ TWEEN 80®, and 0.75% w/v Span 85.TM. and optionally MTP-PE. Yet another submicron oil-in-water emulsion is MF75, also known as SAF, containing 10% squalene, 0.4% ~~Tween 80™~~ TWEEN 80®, 5% pluronic-blocked polymer L121, and thr-MDP, also microfluidized into a submicron emulsion. MF75-MTP denotes an MF75 formulation that includes MTP, such as from 100-400 µg MTP-PE per dose.

Delete the last paragraph on page 56 and replace it with:

The invention may also comprise combinations of aspects of one or more of the adjuvants identified above. For example, the following adjuvant compositions may be used in the invention:

- (1) a saponin and an oil-in-water emulsion (WO99/11241);
- (2) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g. 3dMPL) (see WO94/00153);
- (3) a saponin (e.g., QS21) + a non-toxic LPS derivative (e.g. 3dMPL) + a cholesterol;
- (4) a saponin (e.g. QS21) + 3dMPL+IL-12 (optionally + a sterol) (WO98/57659);
- (5) combinations of 3dMPL with, for example, QS21 and/or oil-in-water emulsions (See European patent applications 0835318, 0735898 and 0761231);
- (6) SAF, containing 10% ~~Squalene~~ squalene, 0.4% ~~Tween 80~~ TWEEN 80®, 5% pluronic-block polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion[.];
- (7) ~~Ribi™~~ RIBI™ adjuvant system (RAS), (Ribi Immunochem) containing 2% Squalene, 0.2% ~~Tween 80~~ TWEEN 80®, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL+CWS (~~Detox™~~ DETOX™);
- (8) one or more mineral salts (such as an aluminum salt) + a non-toxic derivative of LPS (such as 3dPML); and
- (9) one or more mineral salts (such as an aluminum salt) + an immunostimulatory oligonucleotide (such as a nucleotide sequence including a CpG motif).

Delete the first paragraph on page 57 and replace it with:

Aluminum salts and ~~MF59~~ MF59® are preferred adjuvants for use with injectable influenza vaccines. Bacterial toxins and bioadhesives are preferred adjuvants for use with mucosally-delivered vaccines, such as nasal vaccines.

Delete the first full paragraph on page 63 and replace it with:

*In silico analyses:* All the 894 protein coding genes and the corresponding peptide sequences encoded by the *C. trachomatis* genome UW-3/Cx (Stephens et al., 1998. Science 282: 754-9) were retrieved from the National Center for Biotechnology Information web site (~~http://www.ncbi.nih.gov/~~). Putative surface exposed proteins were selected primarily on the basis of GenBank annotation and sequence similarity to proteins known to be secreted or surface-associated. Sequences annotated as hypothetical, which typically lack significant homologies to well characterized proteins, were analyzed for the presence of leader peptide and/or transmembrane regions with PSORT algorithm (Gardy et al., Nucleic Acids Res. 2003 Jul 1;31(13):3613-7). Following these criteria, a set of 158 peptides were selected for expression and in vitro screening.

Delete the first full paragraph on page 65 and replace it with:

For Western blot analysis, total proteins from purified *C. trachomatis* GO/96 serotype D EBs (2  $\mu$ g [[ug]] per lane) were separated by SDS-PAGE and electroblotted onto nitrocellulose membranes. After 30 min. of saturation with PBS-dried skimmed milk (5% w/v) membranes were incubated overnight with preimmune and immune sera (standard dilution 1:400) and then washed 3x with phosphate-buffered saline (PBS)-~~Tween-20~~ TWEEN 20® (0.1% v/v). Following a 1 hour incubation with a peroxidase-conjugated anti-mouse antibody (final dilution 1:5,000 Amersham;) and washing with PBS-TWEEN® ~~PBS-Tween~~, blots were developed using an Opti-4CN Substrate Kit (Bio-Rad).

Delete the second full paragraph on page 65 and replace it with:

Analyses were performed essentially as previously described (See Montigiani et al., *supra*). Gradient purified, heat-inactivated GO/96 serotype D EBs (2x10<sup>5</sup> cells) from *C. trachomatis* resuspended in phosphate-saline buffer (PBS), 0.1% bovine serum albumin (BSA), were incubated for 30 min. at 4°C with the specific mouse antisera (standard dilution 1:400). After centrifugation and washing with 200 µl of PBS-0.1% BSA, the samples were incubated for 30 minutes at 4°C with Goat Anti-Mouse IgG, F(ab)'2-specific, conjugated with R-Phycoerythrin (Jackson ImmunoResearch Laboratories Inc.). The samples were washed with PBS-0.1% BSA, resuspended in 150 µl of PBS-0.1% BSA and analysed by Flow Cytometry using a FACSCALIBUR® ~~FACSCalibur~~ apparatus (Becton Dickinson, Mountain View, Calif.). Control samples were similarly prepared. Positive control antibodies were: i), a commercial anti-*C. pneumoniae* specific monoclonal antibody (Argene Biosoft, Varilhes, France) and, ii), a mouse polyclonal serum prepared by immunizing mice with gradient purified *C. trachomatis* EBs.

Delete the last full paragraph on page 65 and replace it with:

Background control sera were obtained from mice immunized with the purified GST or HIS peptide used in the fusion constructs (GST control, HIS control). FACS data were analysed using the CELLQUEST® software ~~Cell Quest Software~~ (Becton Dickinson, Mountain View, Calif.). The significance of the FACS assay data has been elaborated by calculating the Kolmogorov-Smirnov statistic (K-S score.) (See Young, I. T. 1977. *J Histochem Cytochem* 25:935-41). The K-S statistic allows determining the significance of the difference between two overlaid histograms representing the FACS profiles of a testing protein antiserum and its relative control. All the proteins that showed a K-S score higher than 8.0 have been listed as FACS positive, being the difference between the two histograms statistically significant (p<0.05). The D/s(n) values (an index of dissimilarity between the two curves) are reported as “K-S score” in Tables 1(a) and 1(b).

Delete the last paragraph on page 75 and replace it with:

*Mouse Model for in-vivo screening for CT protective antigens:* A Mouse Model of *Chlamydia trachomatis* (CT) genital infection for determining in-vivo protective effect of CT antigens (resolution of a primary *Chlamydia* infection) was used. The model used is described as follows: Balb/c female mice 4-6 weeks old were used. The mice were immunized intraperitoneally (ip) with a mixture of two recombinant CT antigens in the groups as set out in Table 2 below. These CT antigens were determined to be FACS positive and/or neutralizing (see Table 1(a)). Three doses of the CT antigen mixture were given. The CT antigens in Groups 1 and 2 were HIS fusion proteins. The CT antigens used in Group 3-6 were GST fusion proteins. The mice were given hormonal treatment 5 days prior to challenge with 2.5 mg of DEPROVERA® ~~DepoProvera~~ (medroxyprogesterone acetate).

On page 77, delete the paragraph at lines 11-16 and replace it with:

Results for 1x5 combos + CFA/AlOH + CpG: FIGS. ~~8(a)-8(d)~~ 8(a)-8(c) show the results obtained after administration of a combination of five different CT antigens (CT045, CT089, CT396, CT398 and CT381) with complementary immunological profiles which demonstrate that this five antigen mix is capable of providing protection against CT challenge in a mouse model of Chlamydial genital infection when used in combination with an immunoregulatory agent, such as AlOH and CpG.

Delete Figures 7 and 9 and replace them with the Replacement Drawings that accompany this paper.